DOCKET NO.: 213502US0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

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SERIAL NO: 09/944,079 :

FILED: SEPTEMBER 4, 2001 : GROUP ART UNIT: 1651

FOR: AGENT FOR PREVENTING, IMPROVING OR TREATING HYPERTENSION :

#10
8/15/02

DECLARATION UNDER 37 C.F.R. §1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Now comes Atsushi Suzuki who deposes and states:

1. That I am a graduate of Shizuoka University and received my Master's degree in the year 1989.

2. That I have been employed by Kao Corporation for 13 years as a researcher in the field of biological science.

3. That the experimental data presented below were obtained by me or under my direct supervision and control.

4. The experimental data (Table 1) show that the administration of a combination of chlorogenic acid and an organic acid (Vitamin C) having a molecular weight ranging from 60-300 (the elected species) provides superior antihypertensive effects than the administration of chlorogenic acid or the organic acid (Vitamin C) alone. Table 1 also shows the superior

antihypertensive effects exerted by the combination of caffeic acid (a nonelected species) and the organic acid (Vitamin C).

5. Evaluation of the antihypertensive effect of the combination of chlorogenic acid and Vitamin C on rats.

Table 1: Change in systolic blood pressure at 1, 6, 12 and 24 hours after administration of control or test compositions.

	1h	6h	12h	24h	ANOVA
Control Group (saline)	-0.7 ± 2.1	-1.3 ± 1.0	-2.2 ± 1.1	-1.4 ± 1.8	
Test Group I (Vitamin C)	-0.9 ± 2.2	-1.0 ± 1.5	0.5 ± 1.7	-0.6 ± 2.2	
Test Group II (chlorogenic acid)	-1.7 ± 2.1	-6.7 ± 1.8	-6.8 ± 2.3	-3.0 ± 2.8	
Test Group III (caffeic acid)	-4.3 ± 2.5	-2.4 ± 1.9	1.4 ± 1.9	0.1 ± 3.1	
Test Group IV (chlorgenic acid + Vitamin C)	**-3.3 ± 2.9**	**-7.0 ± 1.0**	**-7.6 ± 3.2**	**-6.1 ± 1.5**	*
Test Group V (caffeic acid + Vitamin C)	-5.1 ± 2.1	-5.2 ± 2.6	-4.3 ± 1.6	-3.5 ± 1.8	*

a) results shown as mean value ± standard error (n = 3 to 6)

b) *: indicates a significant difference compared to Test Group I as measured by the ANOVA test at a significance level of 5%.

6. As shown in Table 1, the combination of chlorogenic acid and the organic acid (Vitamin C) produced an immediate effect on reducing high blood pressure (see e.g., values for 1 hour) and sustained this effect over a 24 hour period (see e.g., values for 6, 12 and 24 hours), as compared to the organic acid (Vitamin C) alone. Treatment with chlorogenic acid alone did not produce a significant immediate effect, nor one that was sustainable for 24 hours. Similarly, the combination of caffeic acid and an organic acid (Vitamin C) exhibited a

significant immediate and sustainable effect on systolic blood pressure, compared to caffeic acid or the organic acid alone.

7. The experimental data reported above in Table 1 were obtained using the following materials and methods.

8. Animal handling and blood pressure testing. The blood pressure of each 14 week old male spontaneous hypertensive rat ("SHR") was preliminarily, continuously measured for 5 days by means of a commercially available non-invasive sphygmomanometer (manufactured by Softlon Co.), thereby fully accustoming the rats to the sphygmomanometry, and an evaluation test was then started. All the rats were bred in a breeding chamber in a rat zone under conditions of a temperature of 25°C ± 1°C, a relative humidity of 55 ± 10% and a lighting time of 12 hours (from 7 a.m. to 7 p.m.).

9. Administration of control and test compositions. Physiological saline was administered to the Control Group. In Test Group 1, a solution containing Vitamin C (200 mg/kg) dissolved into physiological saline was administered. In Test Group 2, a solution with chlorogenic acid (50 mg/kg) dissolved into physiological saline was used. In Test Group 3, a solution with caffeic acid (50 mg/kg) dissolved into physiological saline was used. In Test Group 4, a solution with chlorogenic acid (50 mg/kg) and Vitamin C (200 mg/kg) dissolved into physiological saline was administered. In Test Group 5, a solution with caffeic acid (50 mg/kg) and Vitamin C (200 mg/kg) dissolved into physiological saline was administered. Oral administration of each solution was performed evenly in a quantity of 10 ml/kg.

10. Testing method. Each group contained 3 to 6 spontaneously hypertensive rats ("SHR") that were 15 weeks old.. The systolic blood pressure of a tail artery of each rat was

measured before administration of the control or test solution, and 1, 6, 12 and 24 hours after administration.

11. Statistical processing method. The test results obtained and shown in Table 1 were analyzed and expressed as the mean variation ratio (%) and standard error to conduct an analysis of variance based on recurrent measurements. A significance level was defined as at most 5%.

12. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

13. Further deponent saith not.

_____ _____

(Signed) (Date)

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DECLARATION

I, Yoshiaki TODAKA of c/o The Patent Corporate Body ARUGA PATENT OFFICE, 3-6, Nihonbashiningyocho 1-chome, Chuo-ku, Tokyo 103-0013 Japan do solemnly and sincerely declare that I well understand both Japanese and English languages and that I believe the attached English version is a true and complete translation of Japanese Patent Application No. 2000-268104 filed on September 5, 2000 in the name of Kao Corporation.

July 8, 2002

Yoshiaki TODAKA

2000-268104

[Document Name] APPLICATION FOR PATENT

[Reference Number] P04301209

[Filed to] Commissioner, Patent Office

[International Classification] A61K 35/78

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[Designation of Fees]
[Number of Advance Payment Register] 011752
[Amount Paid] 21,000Yen

[List of Appended Documents]
[Document Name] Specification 1
[Document Name] Abstract 1

[Request of Identification of Data] Requested

[Document Name] Specification

[Title of the Invention] AGENT FOR PREVENTING, IMPROVING OR TREATING HYPERTENSION

[Claims]

[Claim 1] An agent for preventing, improving or treating hypertension, comprising (a) a compound selected from the group consisting of caffeic acid, chlorogenic acid and ferulic acid, and esters and pharmaceutically acceptable salts thereof, and (b) an organic acid having a molecular weight of 60 to 300 or a pharmaceutically acceptable salt thereof.

[Claim 1] The agent according to Claim 1 for preventing, improving or treating hypertension, wherein the component (b) is selected from the group consisting of fermented products of grains, fruit juices and extracts thereof.

[Detailed Description of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to an agent for preventing, improving or treating hypertension.

[0002]

[Prior Art]

Cardiac diseases such as angina pectoris, myocardial infarction and heart failure, and cerebrovascular diseases such as cerebral infarction, cerebral hemorrhage and subarachnoid hemorrhage very closely relate to hypertension and stand second and third, respectively, in

the Japanese causes of death. According to the basis research (the 1998 year) of the national life by the Ministry of Health and Welfare, the number of patients going to hospital regularly with hypertension is sixty-four per thousand in Japan and stands first in the cause of decease. As a countermeasure against the hypertension, may be mentioned the use of antihypertensive drugs such as diuretics, sympatholytic depressants, vasodilators and angiotensin converting enzyme inhibitors. These drugs are mainly applied to serious patients of hypertension. On the other hand, general treatments aiming at improving life custom, such as dietetic therapy, therapeutic exercise and restriction of smoking and drinking, are widely applied to slight and serious patients of hypertension. Therefore, the importance of general treatments is recognized. Among others, improvement in the custom of eating is said to be important, and there are many foods traditionally said to have a hypotensive effect. Antihypertensive materials derived from food have heretofore been extensively searched, and isolation and identification of active ingredients having a hypotensive effect have been made in large numbers.

[0003]

[Problems Sought for Solution by the Invention]

However, under the circumstances, many of drugs used for the purpose of treating hypertension are satisfactory in effectiveness, whereas patients are heavily burdened

with their side effects, such as tachycardia and bradycardia, existing in no small numbers. With respect to foods said to have a hypotensive effect, or active ingredients thereof, the effectiveness is not always satisfactory. Further, many of them require a long time to develop a hypotensive effect.

It is therefore an object of the present invention to provide an agent for preventing, improving or treating hypertension, which is excellent in safety, does not become a burden in daily intake and has a higher antihypertensive effect.

[0004]

[Means for the solution of the Problems]

The present invention provides an agent for preventing, improving or treating hypertension, comprising (a) a compound selected from the group consisting of caffeic acid, chlorogenic acid and ferulic acid, and esters and pharmaceutically acceptable salts thereof, and (b) an organic acid having a molecular weight of 60 to 300 or a pharmaceutically acceptable salt thereof.

[0005]

[Mode for Carrying out the Invention]

As the component (a) used in the present invention, may be used an extract of a natural substance containing this component, particularly, a plant. Examples of the plant include coffee, onion, radish, lemon, MOROHEIYA, *Cnidium officinale* Makino, *Angelica acutiloba* Kitagawa,

pine, *Captis japonica* Makino, asafetida, sugarcane, corn, barley and rice.

[0006]

Caffeic acid and chlorogenic acid may also be extracted from a plant such as green beans of coffee, leaves of nandin or an immature fruit of apple. For example, an acid obtained by extraction of seeds of *Coffea arabica* LINNE, *Rubiaceae* with hot water or with an aqueous solution of ascorbic acid or citric acid under heating may be used.

[0007]

Ferulic acid is a compound contained, as an ester thereof, in natural substances, particularly plants such as rice and adlay and may be obtained as a purified product from such a plant or a synthesized product industrially obtained. A ferulic ester is obtained in a hydrous ethanol fraction after rice bran oil obtained from rice bran is partitioned with hydrous ethanol and hexane at room temperature under weakly alkaline conditions. Ferulic acid can be obtained by hydrolyzing the ferulic ester obtained by the above-described process with sulfuric acid with heating under pressure and purifying the resultant hydrolyzate or by culturing *Pseudomonas* in a medium containing clove oil from buds and leaves of *Syzygium aromaticum* MERRILL et PERRY, *Myrtaceae* by steam distillation, or eugenol obtained by purifying the clove oil and subjecting the medium to isolation and

purification. When ferulic acid is prepared by chemical synthesis, it may be prepared by, for example, a condensation reaction of vanillin and malonic acid (Journal of American Chemical Society, 74, 5346, 1952).

[0008]

Incidentally, stereoisomers exist in caffeic acid, chlorogenic acid, ferulic acid or pharmaceutically acceptable salts thereof. However, pure stereoisomers or a mixture thereof may be used in the present invention.

[0009]

Esters of caffeic acid, chlorogenic acid and ferulic acid include those naturally contained in natural substances, particularly, plants, those obtained by conversion by a chemical treatment upon extraction and/or fractionation and those chemically modified. Specific examples thereof include esters with an alcohol having 1 to 40 carbon atoms, i.e., ester compounds with a linear or branched alkyl or alkenyl alcohol, allyl alcohol, terpene alcohol, sterol or trimethylsterol, and esters with plant sterol. As with ferulic acid, their corresponding esters of caffeic acid and chlorogenic acid may be used.

[0010]

The solubility of caffeic acid, chlorogenic acid and ferulic acid in water can be improved by providing them in the form of a pharmaceutically acceptable salt, and their physiological effectiveness can be enhanced. Examples of a basic substance used for forming such a salt include

inorganic bases such as alkali metal or alkaline earth metal hydroxides, for example, such as lithium hydroxide, sodium hydroxide, potassium hydroxide, magnesium hydroxide and calcium hydroxide, and ammonium hydroxide; and organic bases, such as basic amino acids such as arginine, lysine, histidine and ornithine, and monoethanolamine, diethanolamine and triethanolamine, with the alkali metal or alkaline earth metal hydroxides being particularly preferred. The agents according to the present invention may be formulated either by preparing such a salt and adding the salt to other components, or by separately adding a salt-forming component and a component to be formed into a salt to other components to react them in the formulation system.

[0011]

Two or more of the above-described compounds may be used in combination as the component (a) according to the present invention.

[0012]

The component (b) used in the present invention is an organic acid having a molecular weight of 60 to 300. Examples of the organic acid include carboxylic acids, hydroxycarboxylic acids, polycarboxylic acids, keto-carboxylic acids and the like from the viewpoint of structure, and specific examples thereof include acetic acid, lactic acid, citric acid, gluconic acid, fumaric acid, α-ketoglutaric acid, succinic acid, glycolic acid,

malic acid, tartaric acid, pyruvic acid and malonic acid.

Those naturally contained in natural substances, particularly, plants, those converted by a chemical treatment upon extraction and/or fractionation and those chemically modified are also included. Examples of those derived from the natural substances include brewed vinegar prescribed in the Japanese Agricultural Standard and extracts thereof. The term "brewed vinegar" as used herein means vinegar made by acetic acid fermentation, and specific examples thereof include grain vinegar using rice or other grains as a raw material, for example, grain vinegar called "black vinegar" made by stationary brewing by a single-stage fermentation making use of brown rice and *koji* as raw materials, fruit vinegar making use of apple, grape or any other fruit, and other brewed vinegar than grain vinegar and fruit vinegar. Fruit juices or extracts thereof may also be used. Specific examples thereof include juices of fruits such as orange, mandarin orange, apple, grape, pineapple, peach, grapefruit, lemon, Japanese pear, pear, Japanese apricot, navel orange, strawberry, passion fruits, melon, lime, guava, apricot, SHIKUWASSHA, kabosu orange, shaddock, iyokan orange, hassaku orange, cranberry, banana, Japanese plum, mango, kiwi fruit, persimmon and ASERORA, mixed juices and concentrates thereof, and extracts thereof with water, ethanol, methanol, acetic acid, chloroform, dichloromethane, ethyl acetate, n-hexane, acetone, benzene,

petroleum ether, ether or the like. Extracts with water or ethanol are particularly preferred.

[0013]

Two or more of these organic acids may be used in combination as the component (b).

[0014]

When the agent according to the present invention for preventing, improving or treating hypertension is used as a medicine, a pharmaceutically acceptable carrier may be added to the above-described active components to prepare an oral or parenteral composition. Forms of the oral composition include tablets, granules, grains, pills, powder, capsules (including hard capsules and soft capsules), troches, chewable preparations and solutions (drinks). On the other hand, forms of the parenteral composition include intravenously administering preparations such as injections, suppositories, and external skin care preparations.

[0015]

When the agent according to the present invention for preventing, improving or treating hypertension is used as a food, examples of the food include liquid foods such as drinks and soup; emulsion or paste foods such as milk and curry; semisolid foods such as jelly and gumi; solid foods such as gum, bean curd and supplement; powdered foods; and oil-containing foods such as margarine, mayonnaise and dressing.

[0016]

The contents of the respective components in the agent according to the present invention for preventing, improving or treating hypertension are preferably 0.001 to 5% by weight (hereinafter indicated merely by "%"), particularly 0.01 to 1% for the component (a) and 0. 0005 to 10%, particularly 0.001 to 6% for the component (b).

[0017]

With respect to the effective dose of the component (a) in the agent according to the present invention for preventing, improving or treating hypertension per day for an adult (body weight: 60 kg), it is preferably ingested in a dose of 0.1 to 5000 mg, particularly 0.5 to 1000 mg in terms of ferulic acid. On the other hand, the component (b) is preferably ingested in a dose of 0.1 to 5000 mg, particularly 0.5 to 1000 mg per day in terms of citric acid for an adult.

[0018]

[Examples]

Example 1:

Evaluation as to inhibition of the rise of blood pressure:

(1) Animal used:

The blood pressure of each of male spontaneous hypertensive rats (SHR) aged 7 weeks was preliminarily continuously measured for 7 days by means of a commercially available non-invasive sphygmomanometer (manufactured by Softlon Co.) for rat, thereby fully

accustoming the rats to the sphygmomanometry, and an evaluation test was then started. All the rats were bred (in a breeding chamber in a rat zone) under conditions of a temperature of 25 ± 1°C, a relative humidity of 55 ± 10% and a lighting time of 12 hours (from 7 a.m. to 7 p.m.).

[0019]

(2) Administration method and dosage:

In Control Group, drinking water and a commercially available powdered feed were freely ingested. In Comparative Group, a 0.1% aqueous solution of citric acid was used as drinking water, and a commercially available powdered feed was freely ingested. In Test Group, an aqueous solution containing 0.1% of citric acid and 0.1% of ferulic acid was used as drinking water, and a commercially available powdered feed was freely ingested.

[0020]

(3) Testing method:

Eight SHRs were used as a group. The systolic blood pressure of a tail artery of each rat was measured after 4 weeks from the beginning of the administration.

[0021]

(4) Statistical processing method:

The thus-obtained measurement results were expressed by a mean and standard error to conduct a Student's t-test. A level of significance was defined as at most 5%.

[0022]

The systolic blood pressures in each group before

the administration and after 4 weeks from the administration are shown in Table 1. As apparent from Table 1, a marked inhibitory effect on the rise of blood pressure was observed by the ingestion of Test Group.

[0023]

[Table 1]

	Systolic blood pressures (mmHg)	
	Before administration	After 4 weeks from administration
Control Group	148.1 ± 3.2	195.0 ± 4.3
Comp. Group	148.8 ± 3.8	190.7 ± 3.4
Test Group	148.6 ± 4.0	182.6 ± 3.1 *

*: There is a significant difference at a significance level of at most 5% as against Control Group.

Each value is expressed by mean ± standard error.

[0024]

Example 2: Evaluation as to immediate hypotensive effect:

(1) Animal used:

Male spontaneous hypertensive rats (SHR) aged 14 weeks were preliminarily bred in the same manner as in Example 1.

[0025]

(2) Administration method and dosage:

In Control Group, water was orally administered. In Comparative Group 1, a 0.1% aqueous solution of citric acid was orally administered. In Comparative Group 2, a

0.2% aqueous solution of ferulic acid was orally administered. In Test Group, an aqueous solution containing 0.1% of citric acid and 0.2% of ferulic acid was orally administered. The dose was determined to be 15 mL/kg.

[0026]

(3) Testing method:

Six SHRs were used as a group. The systolic blood pressure of a tail artery of each rat was measured after 1 hour from the beginning of the administration.

[0027]

(4) Statistical processing method:

The thus-obtained measurement results were expressed in the same manner as in Example 1.

[0028]

The systolic blood pressures in each group before the administration and after 1 hour from the administration are shown in Table 2. As apparent from Table 2, marked lowering of blood pressure was observed by the ingestion of Test Group.

[0029]

[Table 2]

	Systolic blood pressures (mmHg)	
	Before administration	After 1 hour from administration
Control Group	206.8 ± 3.4	198.0 ± 5.6
Comp. Group 1	206.1 ± 2.6	196.4 ± 3.9
Comp. Group 2	208.0 ± 4.1	179.7 ± 4.4 *
Test Group	207.4 ± 3.3	170.6 ± 2.1 *#

*: There is a significant difference at a significance level of at most 5% as against Control Group.

*#: There is a significant difference at a significance level of at most 5% as against Comparative Group 2.

Each value is expressed by mean ± standard error.

[0030]

Example 3: Soft capsule preparation

Gelatin	70.0%
Glycerol	22.9
Methyl p-hydroxybenzoate	0.15
Propyl p-hydroxybenzoate	0.51
Water	6.44

The soft capsule (oval form, weight: 150 mg) composed of the above composition was charged with 50 mg of ferulic acid and 450 mg of citric acid to prepare a soft capsule preparation.

[0031]

Example 4: Drink

Nonfat milk	3.5%
Lemon extract	3.5
Fructose	9.0
Sodium ferulate	0.1
Citric acid	0.1
Ascorbic acid	0.1
Perfume base	0.1
Water	83.6

The drink of the above-described composition was high in shelf stability, good in flavor and tasty.

[0032]

Example 5: Cookie

Rapeseed oil	15.0%
Corn starch	15.0
Orange extract	5.0
Wheat	50.0
Butter	5.0
Fructose	14.0
Cycloartenol ferulate	1.0
Common salt	0.5
Baking soda	0.5
Water	10.0

Cookie composed of the above-described composition was baked.

[0033]

Example 6: Separate type dressing

Olive oil	40.0%

Wine vinegar	50.0
Common salt	1.25
Pepper	0.3
Sodium caffeate	0.1
Granular mustard	8.35

A separate type dressing composed of the above composition was prepared.

[0034]

Example 7: Tablet preparation

Corn starch	44.0%
Crystalline cellulose	40.0
Ascorbic acid	0.01
Silicic anhydride	0.5
Olive oil	4.49
Citric acid	5.0
Chlorogenic acid	1.0

A tablet preparation composed of the above composition was formulated.

[0035]

[Effects of the Invention]

The agent for preventing, improving or treating hypertension according to the present invention inhibits the rise of blood pressure, improves hypertension and is useful as a medicine for preventing or treating hypertension, and besides, food and drink, food for specific health and a quasi-drug.

[Document Name] Abstract

[Abstract]

[Means for Solution] An agent for preventing, improving or treating hypertension, comprising (a) a compound selected from the group consisting of caffeic acid, chlorogenic acid and ferulic acid, and esters and pharmaceutically acceptable salts thereof, and (b) an organic acid having a molecular weight of 60 to 300 or a pharmaceutically acceptable salt thereof.

[Effects] The agent inhibits the rise of blood pressure, improves hypertension and is useful as a medicine for preventing or treating hypertension, and besides, food and drink, food for specific health and a quasi-drug.

[Selected Figure of Drawings] None